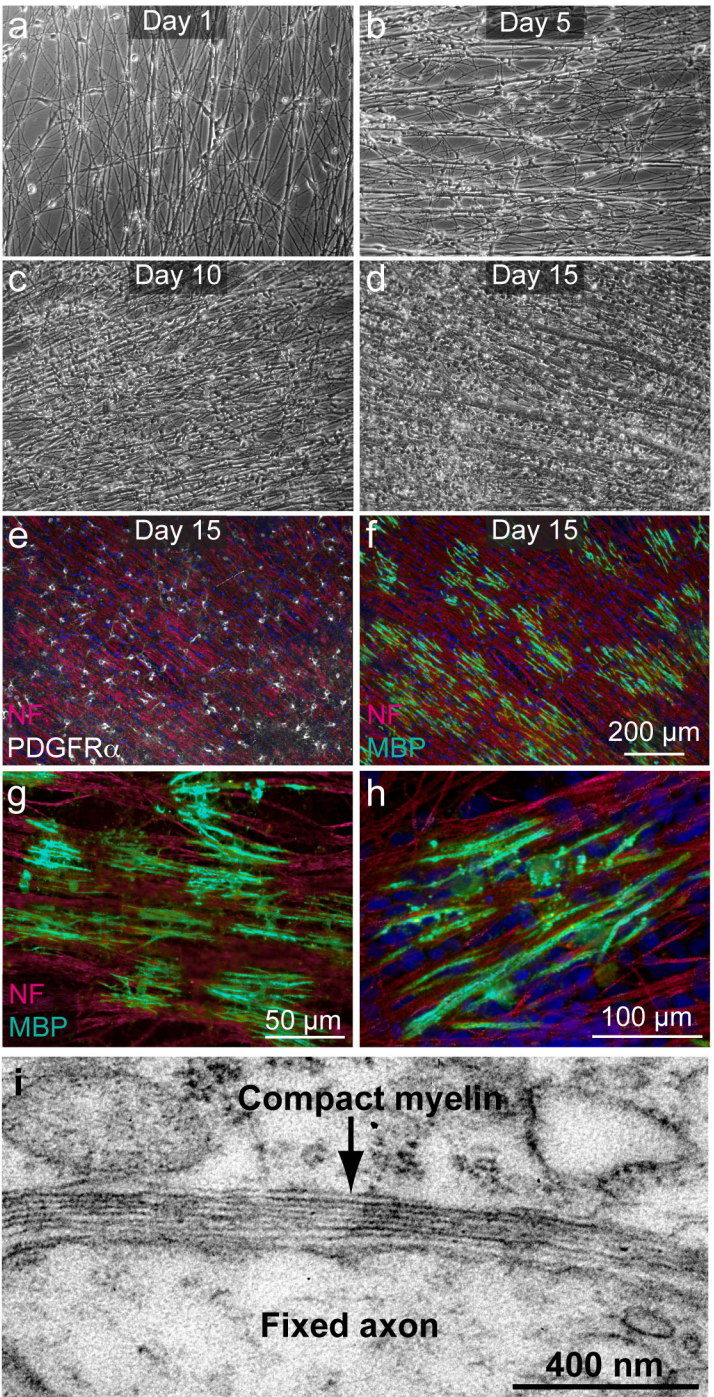
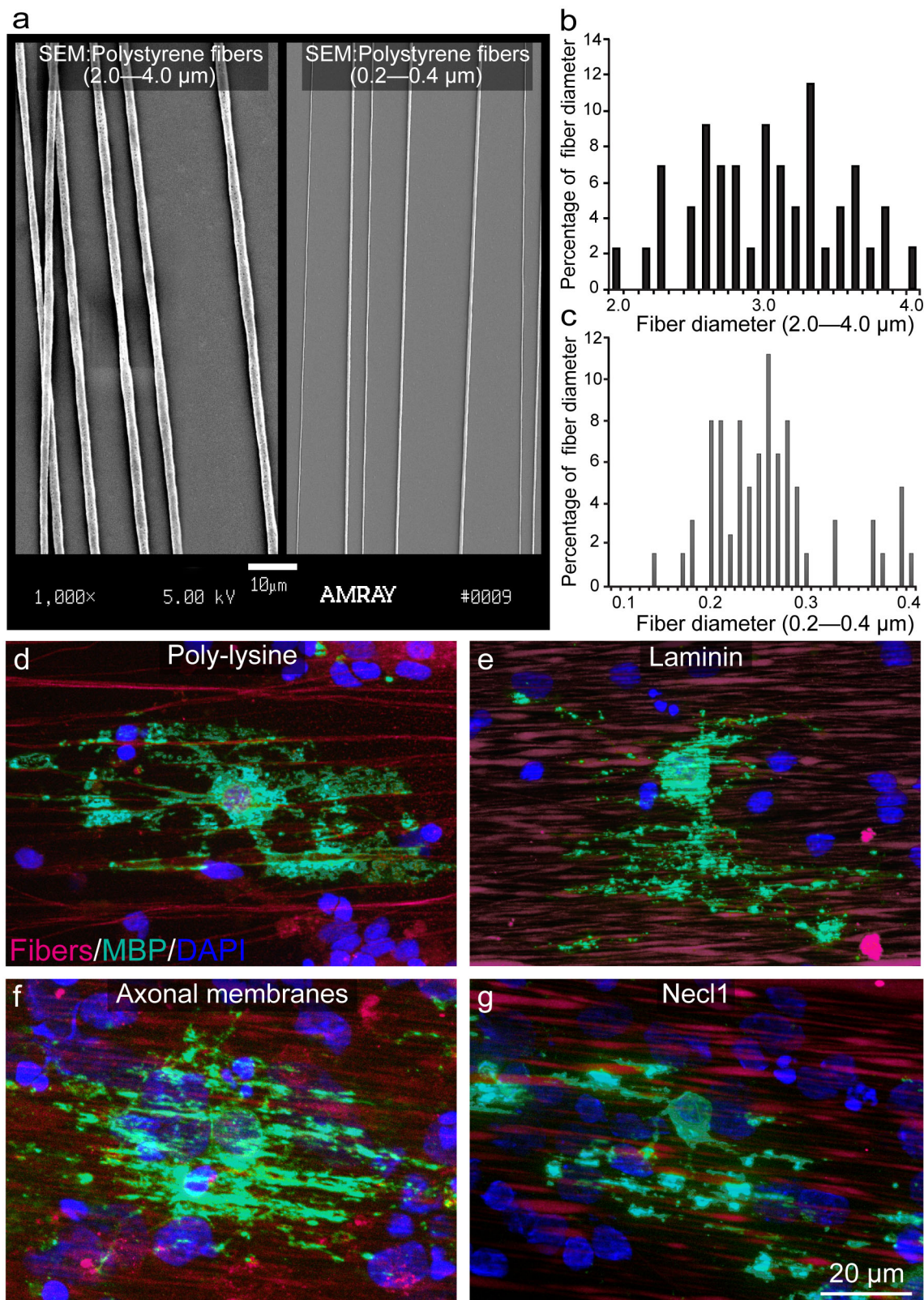


# Supplementary Figure 1



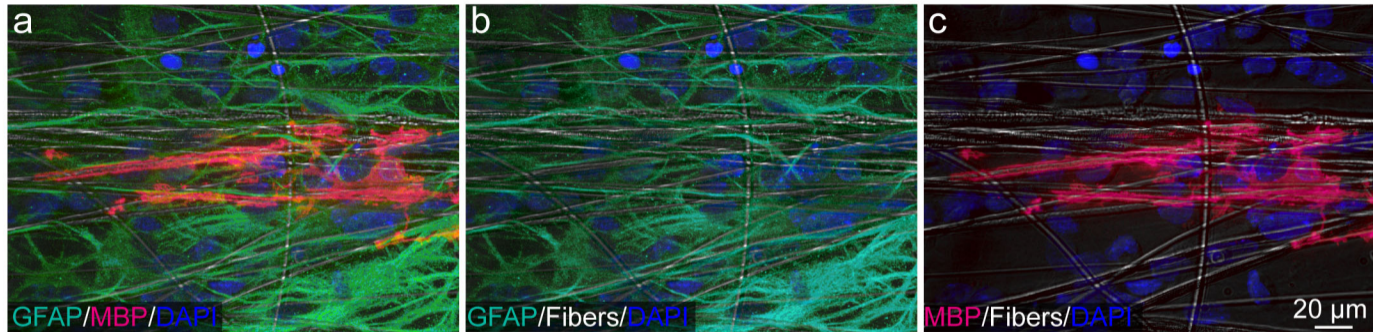
**Supplementary Figure 1** | Oligodendrocytes myelinate axons in the absence of dynamic signaling. (a-d) Phase-contrast images of OPCs cultured on DRG axons over 15 days in vitro (DIV). (e-h) Immunostaining of OPCs cultured on DRGs for 15 DIV illustrates differentiation and myelination. Axons are identified by immunostaining for neurofilament (NF, magenta), OPCs are identified by PDGFR $\alpha$  (white) and myelinating oligodendrocytes are identified by immunostaining for MBP (turquoise). Nuclei are identified with DAPI (blue). Images of oligodendrocytes myelinating (f) live axons and (g, h) fixed axons alike. (i) Electron micrograph of the cultures at 15 DIV showing the formation of compact myelin on a fixed axon. Scale bars, 200  $\mu$ m (f), 50  $\mu$ m (g), 100  $\mu$ m (h), 400 nm (i).

# Supplementary Figure 2



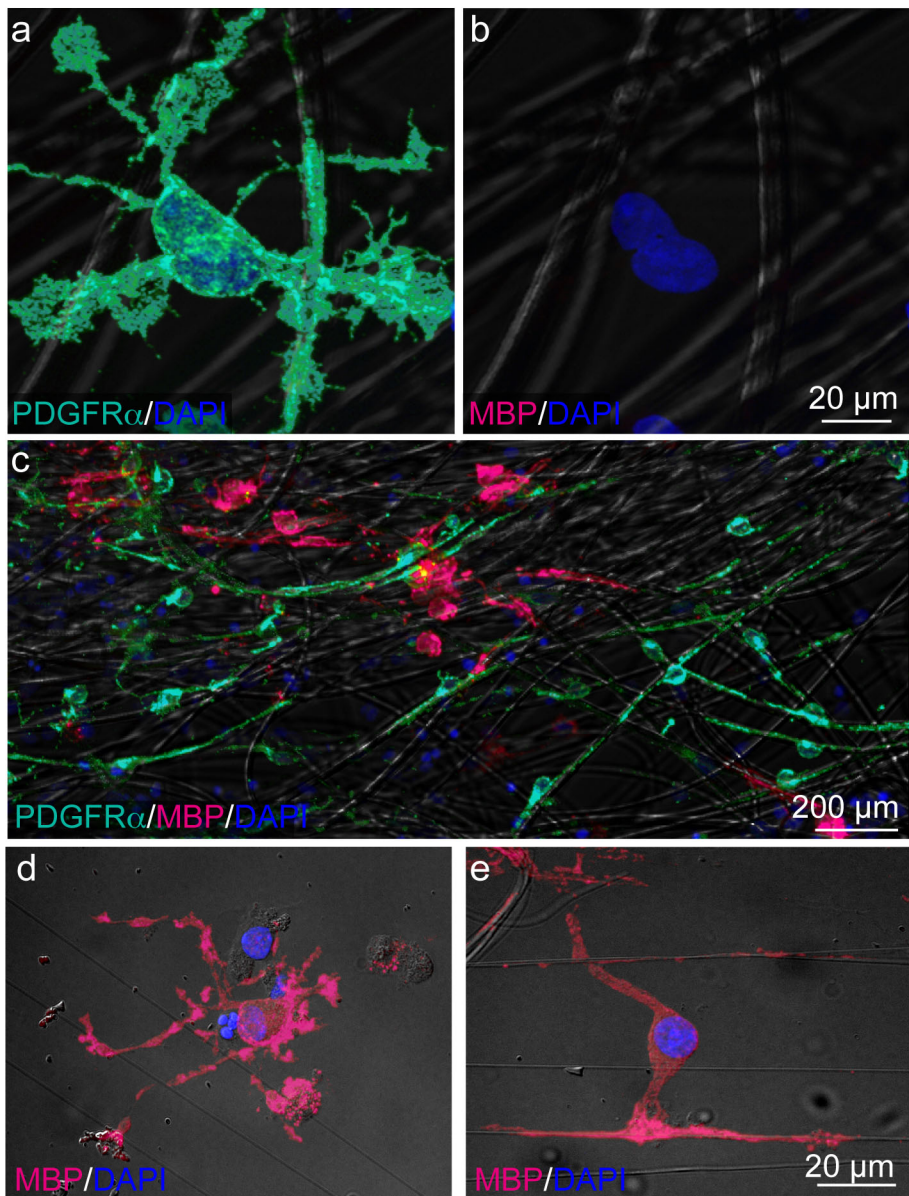
**Supplementary Figure 2** | Cell adhesion molecules and axonal membranes do not overcome the minimum diameter threshold. (a) Images from scanning electron microscopy demonstrating the overall quality and distribution of the nanofibers. Images were used to quantify the distribution of fiber diameters aligned on coverglasses. (b, c) Graphs illustrate representative fiber diameter distributions of the (b) large diameter (2.0—4.0  $\mu\text{m}$ ) fiber range and the (c) small diameter (0.2—0.4  $\mu\text{m}$ ) fiber range. (d—g) Images of MBP+ oligodendrocytes cultured on small diameter fibers at 15 DIV. Fibers were coated with (d) poly-L-lysine, (e) laminin, (f) axonal membranes and (g) Nect1. As it is difficult to visualize small diameter fibers by phase contrast microscopy, the fibers are labeled with rhodamine to allow for clear visualization. Scale bar, 20  $\mu\text{m}$ .

## Supplementary Figure 3



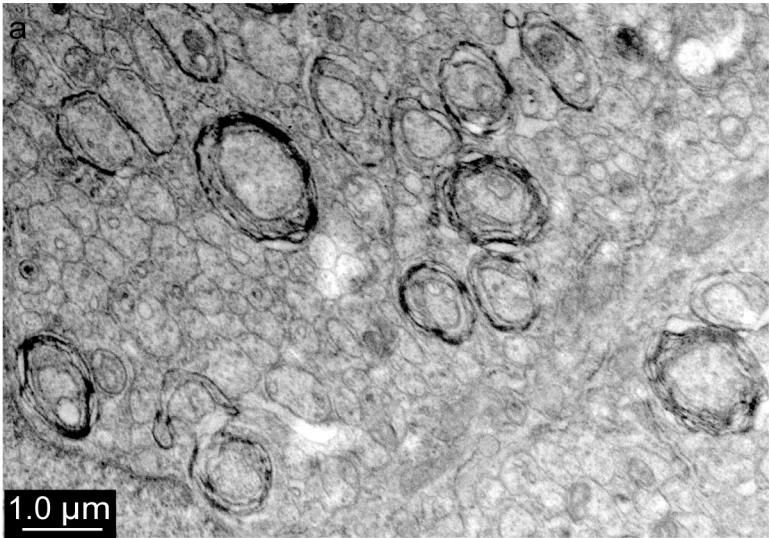
**Supplementary Figure 3** | (a-c) Images of OPCs co-cultured with astrocytes on large diameter fibers at 15 DIV. (a) An oligodendrocyte immunostained against MBP and GFAP. (b) Separate panels illustrating astrocytes on fibers (GFAP+, turquoise) extend their long fibrous membrane processes across and/or along the fibers while (c) oligodendrocytes (MBP+, magenta) form myelin-like segments around the fibers. Scale bar, 20  $\mu\text{m}$ .

## Supplementary Figure 4

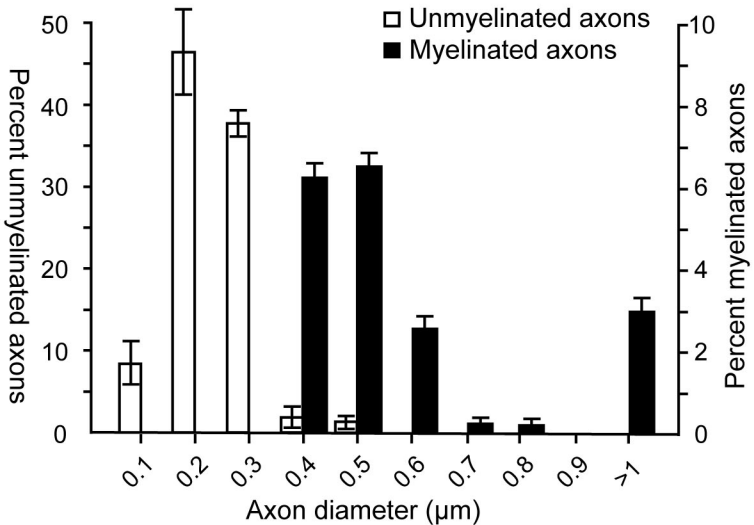


**Supplementary Figure 4** | OPCs ensheath fibers prior to differentiation and MBP expression. (a, b) Immunostaining of OPC-fiber cultures at 5 DIV reveals PDGFR $\alpha$ + segments made by an OPC on fibers in the absence of MBP expression. (c) Low magnification image of multiple PDGFR $\alpha$ +/MBP-segments on fibers reveal their morphological similarities to MBP+/PDGFR $\alpha$ -segments made by oligodendrocytes. (d, e) Images of acutely purified mature MBP+ oligodendrocytes cultured (d) on small diameter fibers and (e) on large diameter fibers demonstrating that mature cells maintain a similar sensitivity to fiber diameter as OPCs and cannot wrap fibers below the minimum diameter threshold. Scale bar, 20  $\mu$ m (a, b), 200  $\mu$ m (c), 20  $\mu$ m (d, e).

# Supplementary Figure 5

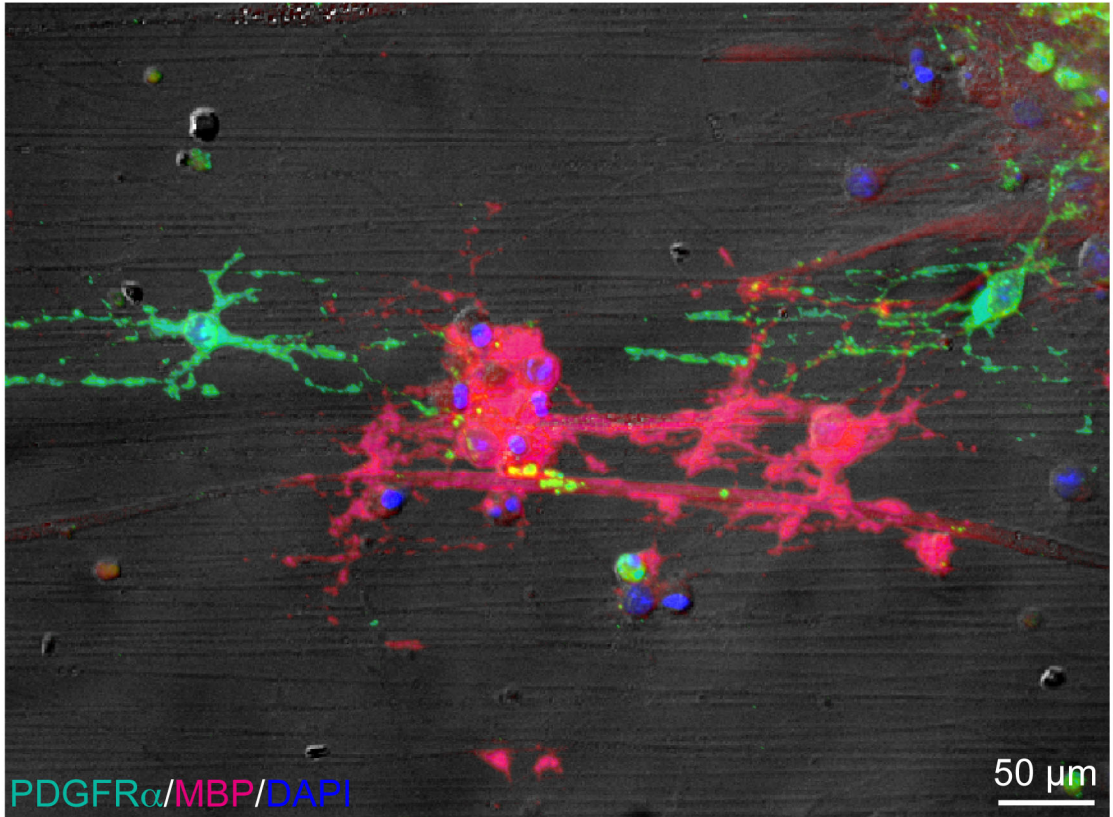


b



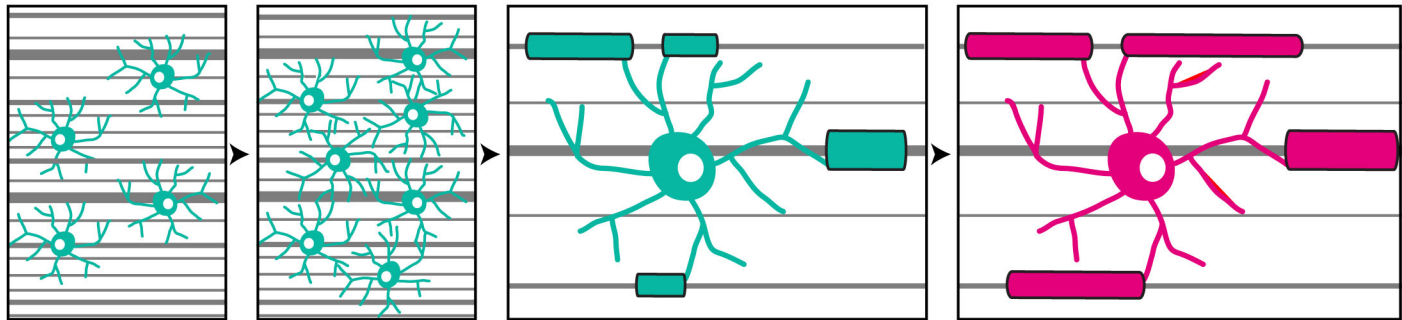
**Supplementary Figure 5** | Preference for larger diameter axons during developmental myelination of optic nerves. (a) Electron micrograph of an optic nerve demonstrating large diameter axons already engaged in myelination at postnatal day 8. (b) Quantification of the unmyelinated and myelinated axons as a function of axon caliber. ( $n = 3$ , mean of three independent experiments in which 300 axons were counted for quantification). Error bars represent SD. Scale bar, 0.5  $\mu\text{m}$  (a).

## Supplementary Figure 6



**Supplementary Figure 6** | Preference for larger diameter fibers by oligodendroglia. Immunostaining images of 15 *DIV* OPCs and oligodendrocytes cultured on mixed diameter fibers. A small percentage of large diameter fibers (2.0 - 4.0  $\mu\text{m}$ ) are present, while the majority of the fibers in the culture are small diameter (0.4—0.8  $\mu\text{m}$ ). These small diameter fibers are still above threshold. OPCs (in turquoise) are immunostained with PDGFR $\alpha$ , oligodendrocytes (in magenta) with MBP, and nuclei with DAPI. Scale bar, 50  $\mu\text{m}$ .

## Supplementary Figure 7



**Supplementary Figure 7** | Depiction of a novel chronology of events during developmental myelination. OPCs (first panel) proliferate and migrate along fibers until reaching a critical density while sampling different fibers (second panel). OPCs begin to select and ensheath fibers with diameters greater than  $0.3 \mu\text{m}$  prior to differentiation into MBP<sup>+</sup> oligodendrocytes (third panel). Concomitantly, OPCs undergo differentiation, express myelin proteins and wrap the fibers (fourth panel).